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Combination therapy of cyclosporine A and mycophenolate mofetil in rats with adriamycin-induced nephrotic syndrome

Combination theraphy in experimental nephrotic syndrome

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Abstract

Aim: The lack of effective treatment for steroid-resistant nephrotic syndrome has led to the search for new treatments and the emergence of combined therapies. In our study, we aimed to reduce side effects and increase effectiveness by combination with low-dose cyclosporine and MMF.

Material and Methods: Fifty Wistar albino rats were included in our study. Five groups were formed as follows: untreated nephrotic syndrome, cyclosporine, MMF, low-dose cyclosporine, and a combined therapy group. Nephrotic syndrome parameters were studied. Rats were sacrificed at the end of the study. Glomerulosclerotic index, interstitial fibrosis score, and total damage score were calculated with histopathological examination.

Results: Urine protein/creatinine significantly decreased in the MMF and low-dose CsA groups after treatment. Serum total protein and albumin levels were preserved better in the combined therapy group. The control of hyperlipidemia was better in the combined therapy group than the others. The best results for oxidative damage were detected in the low-dose CsA and combined therapy groups. Serum creatine was detected to be higher in the CsA group than in other groups. The glomerulosclerotic index and total damage score were found to be significantly higher in the CsA group compared to other groups. The interstitial fibrosis score was found to be significantly higher in the MMF group compared to the combined therapy group. TGF-β was significantly higher in the MMF group compared to the CsA group. Osteopontin was significantly lower in the CsA group than the other groups.

Discussion: This study showed that while MMF and low dose CsA have similar successful effects in protecting serum protein and creatinine, oxidative stress can be kept lower by using low-dose CsA, and hyperlipidemia can be suppressed more successfully with combined therapy.

Keywords

Nephrotic syndrome; Cyclosporine A; Mycophenolate mofetil

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Introduction

In children, nephrotic syndrome is a common and one of the leading disorders that cause chronic kidney failure. Although various treatments and numerous drugs are used in the treatment of steroid- resistant nephrotic syndrome (SRNS), there is still no effective and definitive treatment. In addition, the serious side effects of the drugs used make it necessary to seek new treatment options. Cyclosporine A is the only drug that has shown efficacy in the treatment of steroid- resistant nephrotic syndrome, but its various side effects, especially nephrotoxicity, limit its use and cause difficulties in treatment. For this reason, various drugs and combined therapies have emerged in the treatment of SRNS. Mycophenolate mofetil (MMF) is a drug that has been used effectively in the treatment of steroid- dependent nephrotic syndrome. In previous studies, antiproteinuric efficacy was demonstrated and fewer side effects were found compared to cyclosporine A treatment [1-4]. In the pathogenesis of SRNS, it has been reported that with chronic severe proteinuria, with the effect of treatments such as cyclosporine A, an increase in free oxygen radicals due to oxidative stress in the kidney tissue, with the development of fibrosis in the kidney tissue as a result of a series of immune reactions, including the concentration of cytokine production at the tissue level, and the progress of fibrosis to sclerosis give rise to the development of end-stage renal failure [5]. A decrease in proteinuria, crescent formation and glomerulosclerosis has been demonstrated in glomerulonephritis rat models treated with MMF. Inhibition in macrophage and osteopontin expression has been shown [6]. In recent years, few case studies have been reported that have used a combination of CsA and MMF [7-9].

Adriamycin-induced nephrotic syndrome is a relevant experimental model [10].

This experimental model was used in our three previous studies [11,12]. In one of our studies, a significant decrease in proteinuria with cyclosporine A was demonstrated, but impairment of kidney function in rats with adriamycin-induced nephrotic syndrome. On the other hand, MMF moderately reduced proteinuria and did not cause impairment in renal functions (Baysal YE. Effects of Cyclosporin A, Mycophenolate Mofetil, Vitamins A, D, E and N-acetylcysteine in Adriamycin-induced nephrotic syndrome. Akdeniz University Faculty of Medicine, Pediatric Nephrology Minor Thesis, Antalya 2008). Based on this, we thought that the combination of low-dose cyclosporine A and MMF could be more effective and have fewer side effects.

Material and Methods

Fifty adult, male, Wistar albino rats were included in our study. Rats were divided into 5 groups, with 10 rats in each group. They were grouped as an untreated group with adriamycin-induced nephrotic syndrome (group A), cyclosporine A at a dose of 25 mg/kg (Group B), mycophenolate mofetil at a dose of 20 mg/kg/day (Group C), low-dose (10 mg/kg/d) cyclosporine A (Group D) and the combined therapy group (low-dose cyclosporine A + mycophenolate mofetil Group E). Akdeniz University Experimental Animals Ethics Committee approval was obtained for this study.

Rats were fed standard rat chow and water during the experimental process. No diet and water restrictions were applied. All rats were weighed before treatment, 24-hour urine samples were collected, blood samples were taken and adriamycin at a dose of 2mg/kg was administered into the tail vein under ether anesthesia, and a second dose injection of adriamycin was applied on day 21. Treatments were started after the second dose of adriamycin injection. The weights of all rats were measured once a week. Blood pressures were measured from the tail arteries under mild ether anesthesia using a non-invasive method (tail-cuff) once a month. The signals received by the ring-shaped pressure probe attached to the tail were transferred to the computer via the MP 100A-CE data acquisition system (BIOPAC Systems, CA-USA) and the MAY-BPHR200 unit, and the measurements were done with the pressure traces drawn with the "Acknowledge" package program. After the determination of the baseline measurements of all animals before the experiment, blood pressure monitoring was continued with the measurements performed once a month until the end of the experiment. The average of the successfully measured blood pressure levels in each rat was recorded three times.

Blood samples and 24-hour urine samples were taken at baseline, at weeks 4 and 8. Serum and urine samples were stored at -70 degrees until the end of the study. Serum total protein, albumin, creatinine, total cholesterol, triglyceride, total oxidant level (TOS) and total antioxidant status (TAS) were studied from blood samples. Total protein and creatinine were studied from 24-hour urine samples. Based on the data obtained, creatinine clearance, urine protein/creatinine ratio, 24-hour urine protein and oxidative stress index were calculated.

Serum and urine creatinine levels were measured in a Modular PPP autoanalyzer (Roche Diagnostics, GmbH, Mannheim) using the "rate-blanked and compensated" Jaffe method with Roche kits. Serum total protein and albumin levels were measured spectrophotometrically using Roche kits in Modular PPP autoanalyzer (Roche Diagnostics, GmbH, Mannheim). Serum total cholesterol and triglyceride levels were measured by enzymatic spectrophotometric method using Roche kits in a Modular PPP autoanalyzer (Roche Diagnostics, GmbH, Mannheim). The urine total protein levels were measured by turbidimetric method using original Roche kits in a Modular PPP autoanalyzer (Roche Diagnostics, GmbH, Mannheim). Serum Total Oxidant Status (TOS) and Total Antioxidant Status (TAS) analyses were performed spectrophotometrically using the method of Erel. All measurements were made automatically using a V-Twin autoanalyzer (Dade Behring, Syva, Marburg, Germany).

At the end of the study, all rats were sacrificed, their kidneys were removed and weighed. It was then fixed with 6% neutral formalin and covered with paraffin.

Pathology examples

After the hematoxylin- eosin, Masson's trichrome, TGF-Beta, osteopontin staining; GSI (glomerulosclerotic index), IFS (interstitial fibrosis score) scores were calculated. TGF-staining for intersticium and OPN (osteopontin) staining for tubules were evaluated. Grade 1+ indicated pathological involvement of 25% of a glomerulus, 2+ indicated 25–50%, 3+ indicated 50–75% and 4+ indicated 75–100% (Figure 1). The glomerular sclerosis index was calculated by taking the average of the total score

of 20 glomeruli for each sample. For instance, if there is 1 (+) lesion in 12 glomeruli, 2 (+) lesions in 1 glomerulus, 3 (+) lesions in 1 glomerulus, and no damage in 6 glomeruli, 0.85 is obtained by dividing 17, which is the total score, by 20, was calculated as the glomerular sclerosis index. The degree of interstitial fibrosis was determined with the ocular grid using the standard point count method. A 21x21 ocular grid containing 441 points was placed on the ocular part of the light microscope, and the spots falling on the stained area with Masson's trichrome, TGF-β and Osteopontin (OPN) were counted at 40X magnification. The arithmetic mean of the results of 10 consecutive regions that do not overlap in the interstitial area in the biopsy samples was calculated as the interstitial fibrosis scoring.

The data obtained were analyzed using SPSS, percentage, arithmetic mean, chi-square, Mann- Whitney-U and Kruskal-Wallis tests. The mean and standard deviation values were calculated, p <0.05 was considered significant.

Results

The study was planned for 16 weeks, but was terminated at week 9 due to the high loss of rats. Twelve rats, of which 5 rats in the CsA group and 5 rats in the combined treatment group and 2 rats in the low dose CsA group, were exitus. The data of the rats at baseline and at the 4th and 8th weeks after the treatment and the statistical comparisons between the groups are given in Tables 1, 2, 3, respectively.

We demonstrated the development of hypoproteinemia, hypoalbuminemia, and hyperlipidemia, which are laboratory findings of nephrotic syndrome, in all rats at the 4th week after adriamycin administration. However, there was no increase in proteinuria, in the initial urine samples of the rats, proteinuria values were determined higher than the values at the week 4 and 8. Regarding the reason for this, all stages, obtaining samples, storing samples and evaluating the data were reviewed from the beginning, but no reason to explain was found. No changes had been applied in the vital conditions, nutrition and fluid intake of the rats during this period. Serum samples obtained supported the biochemical findings of nephrotic syndrome, therefore no problem was considered regarding adriamycin administration. Since after considering all the reasons, the cause was not found, it was thought that there might be a technical error that we could not explain.

In histopathological evaluation, glomerulosclerosis index was 1.53±0.45, 2.13±0.27, 1.78±0.18, 1.72±0.34, 1.65±0.26 in all groups, respectively. Glomerulosclerosis index was found significantly higher in the CsA group in comparison with other groups (p, A-B: 0.012, B-C: 0.03, B-E: 0.022). The total damage score was 213.33±27.5 in the cyclosporine A group and had the highest value. It was found 153.5±45.15 in the untreated nephrotic syndrome group, 178±18.58 in the MMF group, 172.5±34.85 in the low dose CsA group, 165±26.22 in the combined therapy group. Total damage score was found to be significantly higher in the CsA group compared to the other groups (p: A-B: 0.012, B-C: 0.03, B-D: 0.011, B-E: 0.022). The degree of TGF-β staining was found significantly higher in the MMF treatment group compared to the CsA treatment group (B-C: 0.044). TGF-β staining was 11.18±3.72 in group A, 9.05±1.12 in group B, 10.57±1.5 in group C, 9.82±1.54 in group D, 9.6±

Table 1. Baseline data of all groups and statistical analysis results between groups

Group	A	B	C	D	E	P
Weight	295±34	300±42	326±25	301±37	286±63	>0,05
Systolic blood pressure	154±9,7	155±3,6	153±9	148±7,4	151±5,5	>0,05
Diastolic blood pressure	72,20±10,8	65,30±5,88	64,20±6,69	65,62±7,73	66,20±4,94	>0,05
24- hour urine volume	11,00±2,59	4,00±3,30	9,55±2,67	7,50±3,66	3,00±2,00	A-D=0,007, A-E=0,004 B-C=0,022, C-E=0,017 A-B=0,015 Other p> 0,05
Serum albumin	4,23±0,23	4,21±0,19	4,14±0,25	4,19±0,26	4,20±0,20	p>0,05
Serum total protein	7,18±0,47	6,96±0,23	6,87±0,23	6,90±0,39	7,02±0,41	A-B=0,02 A-C=0,011 A-D=0,032 Other p> 0,05
Serum cholesterol	81,10±9,50	69,66±6,02	78,80±9,12	76,62±9,12	76,40±7,53	>0,05
Serum triglyceride	62,50±19,81	86,83±12,49	78,10±24,58	67,87±26,90	73,00±21,94	>0,05
Serum creatinin	0,50±0,09	0,39±0,04	0,36±0,03	0,35±0,01	0,38±0,02	A-B=0,001, A-E<0,001 A-C<0,001, B-D=0,027 A-D<0,001 Other p> 0,05
Urine protein	18,04±9,54	6,52±4,90	16,27±5,58	12,39±4,42	4,40±3,41	>0,05
Urine protein/kreatinin	2,00±0,46	3,49±3,44	2,24±0,53	2,34±0,68	1,88±1,12	>0,05
Creatinin clearance	3,59±3,10	4,84±4,50	2,84±2,82	4,32±3,19	4,39±2,13	>0,05
TOS	50,15±23,49	25,67±4,99	26,35±12,29	28,14±9,54	24,24±9,16	A-B= 0,008, -D=0,016 A-C=0,016 A-E=0,004 Other p> 0,05
TAS	2,52±0,72	2,52±0,72	1,81±0,44	1,81±0,26	1,81±0,34	A-C=0,019, A-D=0,049 A-E=0,019, B-C=0,019 B-D=0,049, B-E=0,019 Other p> 0,05
OSİ	1,90±0,41	1,40±0,17	1,40±0,38	1,47±0,29	1,30±0,28	A-B=0,007, A-C=0,023 A-D=0,019, A-E=0,002 Di p> 0,05

Table 2. All groups data and statistical analysis at 4th week

Group	A	B	C	D	E	P
Weight	297± 29,6	282± 35,7	348± 27,6	307± 35,8	291± 56,8	A-C= 0,003 B-C= 0,012 C-D= 0,003 C-E=0,005
Systolic blood pressure	167 ± 5,97	168± 2,20	156± 5,41	165± 4,86	164± 12,01	A-C= 0,001. C-D= 0,005.
Diastolic blood pressure	87±5,79	80±10,60	78±6,84	80±8,93	83±10,39	p>0,05
24-hour urine volume	8,16±3,51	14,33±17,46	8,10±3,21	6,00±3,20	6,00±4,18	p>0,05
Serum albumin	3,01±0,57	3,51±0,29	3,55±0,37	3,49±0,39	4,94±0,96	p>0,05
Serum total protein	6,15±0,38	5,91±0,32	6,29±0,29	6,30v0,39	6,05±0,39	A-B=0,048 B-C=0,005 B-D=0,019
Serum cholesterol	205±142,15	80±17,28	108±31,12	118±31,12	151±50,98	A-B=0,028 A-C=0,03 B-E=0,023
Serum trigıyceride	100±59,05	80±32,69	80±25,73	46±26,38	127±112,33	p>0,05
Serum creatinin	0,33±0,07	0,52±0,06	0,36±0,04	0,38±0,08	0,39±0,12	A-B= 0,001 B-D= 0,007 B-C< 0,001 B-E= 0,028
Urine protein	5,55±4,0	18,29±25,7	12,10±9,95	10,52±16,2	4,39±2,99	p>0,05
Urine protein/creatinin	0,77±0,60	3,17±1,15	1,70±1,47	1,55±2,06	0,73±0,52	A-B=0,007 B-E=0,002
Creatinine clearance	3,33±2,89	3,73±2,65	6,03±7,53	3,30±2,91	4,37±2,84	p>0,05
TOS	5,79±2,45	9,89±9,83	4,84±0,71	3,87±0,82	10,25±13,73	B-D=0,005 C-D=0,013 C-E=0,049 D-E=0,002 A-D=0,041
TAS	1,43±0,06	1,48±0,19	1,39±0,07	1,39±0,06	1,39±0,07	p> 0,05
OSİ	0,39±0,15	0,61±0,49	0,34±0,04	0,29±0,06	0,42±0,10	B-D=0,013 C-D=0,023 D-E=0,003

Table 3. Eighth-week data and the results of statistical analysis of all groups

Group (8. weeks)	A	B	C	D	E	P
Weight	302± 30,1	244± 55,7	368± 32,7	316± 41,8	269± 73,2	A-B= 0,05, C-D= 0,013 A-C= 0,001, C-E= 0,009 B-C= 0,001, B-D= 0,012
Systolic blood pressure	175± 6,79	164± 3,54	162± 4,97	162± 4,63	166± 1,62	A-B= 0,012, A-C= 0,001 A-D= 0,002 A-E= 0,02
Diastolic blood pressure	109±7,50	98±3,20	88±5,65	82±5,84	84±4,70	A-B=0,014, A-D<0,001 A-C<0,001, A-E=0,002 B-C=0,002, B-D=0,003 B-E=0,009
24-hour urine volume	10,38±4,67	12,83±11,68	13,75±6,77	13,25±13,25	12,80±4,08	p>0,05
Serum albumin	1,84±0,37	1,81±0,43	2,34±0,52	2,19±0,70	2,20±0,24	p>0,05
Serum total protein	5,60±0,66	5,16±0,55	5,31±0,44	5,72±0,26	5,86±0,28	B-D=0,032 B-E=0,044 C-D=0,032 C-E=0,031
Serum cholesterol	376,30±134,46	299,83±188,51	242,30±163,01	275,87±163,01	291,00±67,83	p>0,05
Serum triglyceride	357,40±204,35	360,50±175,12	254,00±199,75	247,12±180,23	257,20±38,40	p>0,05
Serum creatinine	0,27±0,16	0,35±0,22	0,30±0,07	0,28±0,09	0,36±0,11	p>0,05
Urine protein	10,45±10,61	8,79±7,25	8,87±4,35	6,84±2,32	5,78±2,00	p>0,05
Urine protein/ creatinine	0,96±0,69	1,91±2,22	0,68±0,58	0,41±0,12	0,36±0,06	p>0,05
Creatinine clearance	4,59±3,32	5,90±4,32	8,88±11,13	4,91±2,47	3,03±0,34	p>0,05
TOS	9,90±6,02	10,69±5,08	10,27±5,84	4,79±2,88	4,63±3,05	A-D=0,041 B-D=0,028 C-D=0,033 C-E=0,037
TAS	1,51±0,15	1,59±0,12	1,56±0,09	1,56±0,08	1,48±0,13	B-D=0,017, C-D=0,003
OSI	0,63±0,32	0,67±0,30	0,64±0,33	0,34±0,20	0,36±0,19	p>0,05

1.14 in group E. The highest score for the interstitial fibrosis were in the MMF and no treatment group (A: 11.95±4.78, C: 11.2±1.85, respectively). The lowest value was found in the combined therapy group (9.12±1.22). The interstitial fibrosis score (IFS) was found significantly lower in the combined therapy group compared to the MMF group (p: C-E: 0.037). The lowest level in the degree of been stained with osteopontin (OPN) was 21.12±0.49 that was found in the CsA group, and it was significantly lower than the other treatment groups (p, B-C: 0.001, B-D: 0.002, B-E: 0.006). In the other groups were 22.33± 2.53, 23.04±0,97, 22.88±0.84, 23±0.33, respectively.

Discussion

The fact of the rat losses in the CsA and combined treatment groups indicates that immunosuppression and toxicity are significant in these groups. Nephrotic syndrome findings, hypoalbuminemia, hypoproteinemia, and hyperlipidemia were shown in all rats at the 4th and 8th weeks, but proteinuria could not be demonstrated due to possible technical error. In this study, weight gain was found significant in the group receiving MMF and low dose CsA treatment, but weight loss was detected in the group with CsA and combined therapy, but it was not statistically significant. In addition, 5 rats died in each of these groups, and significant weight loss was observed in these rats. Similarly, in the study conducted by Baysal et al. (Effects of Cyclosporin A, Mycophenolate Mofetil, Vitamins A, D, E and N-acetylcysteine in Adriamycin-induced nephrotic syndrome. Akdeniz University Faculty of Medicine, Pediatric Nephrology Minor Thesis, Antalya 2008), no significant weight gain was observed, and weight loss was reported in the group receiving CsA treatment. Weight in the MMF group was significantly higher after treatment than in the other groups. Zoja et al. [13] in rats with Heyman nephritis, and Erisir et al. [12] with creating NS in FSGS model with adriamycin, found weight gain in rats according to weeks. However, in both studies, the treatment groups were different from our study. As mentioned in a similar study, significant weight loss was observed in the group receiving CsA treatment in our study, and it was thought to be associated with CsA toxicity. In a similar previous study, no difference was detected in kidney weights between treatment groups [11]. In our study, it was found to be significantly higher only in the MMF group compared to the CsA group. In the experimental study by Okuda et al. [10], they created adriamycin nephropathy and found that rat systolic blood pressures increased after 8 weeks and then remained higher than in the control group. In our study, we found that systolic and diastolic blood pressure increased significantly.

While the significant increase did not continue after the treatment in the low dose CsA and combined treatment group, the significant increase continued in the other groups.

Serum albumin levels decreased in all groups, but there was no significant decrease in the 4th week in the combined treatment group. The best value after treatment was detected in the MMF treatment group. However, the data may not be sufficient because of the early completion of the study and short-term follow-up in observing the effectiveness of the treatments. In previous experimental studies, a re-increase in albumin level was observed in the following weeks (week 12, 16) after treatment. Serum total protein did not continue to decrease after treatment only in the combined therapy group, and the best value was detected in this group. Based on this, we can think that serum total protein can be better preserved with combined therapy. Serum total protein from high to low, respectively: combined therapy> low dose CsA> no treatment control> MMF> CsA.

In our study, serum creatinine and creatinine clearance were found to be significantly higher in the CsA group at the 4th week compared to the other treatment groups. No statistically significant difference was found between the groups. At the end of the study, the lowest value was in the control group without treatment. In the experimental study by Blume et al. [14] performed by creating passive Heyman nephritis in rats, when proteinuria and serum creatinine levels were compared with CsA and MMF, although proteinuria decreased in both treatment groups, serum creatinine levels were found to be higher in rats receiving CsA. In a study by Baysal et al., there was an increase in creatinine in the CsA group and it was thought to be associated with nephrotoxicity. In an experimental study conducted by Takeda et al. [6], a significant improvement in proteinuria was found in rats with crescentic glomerulonephritis after MMF administration. In the eighth week of our study, it was determined that urine protein/creatinine ratios were significantly decreased in the MMF and low dose CsA groups compared to the 4th week.

Serum total cholesterol values were found best in the MMF group, but it was not statistically significant. Similar to the study conducted by Baysal et al., better results were obtained in the MMF group. In addition, serum triglyceride value was best preserved in the combined group at the end of the study.

It has been shown in previous studies that cyclosporine A increases the oxidant level in the renal tissue [5]. However, there is no such study regarding MMF. In our study, the lowest value at the end of TOS treatment was detected in the combined treatment group and was significantly lower than in the MMF group (p= 0.037). In the low-dose CsA group, it was found to be significantly lower than the untreated control, CsA, and MMF groups. With low-dose CsA and combined therapy, TOS was lower than in other treatment groups. At the 4th week, the oxidative stress index was found to be significantly lower in the low-dose CsA group compared to other treatment groups. Baysal et al. found the lowest value in the MMF group, but the dose of CsA in this study was the same as in our high dose group. The low-dose CsA and combined therapy groups were created in our study for the first time in literature. For this reason, it is not possible to compare them exactly.

TGF-β is a potent fibrogenic factor and has a predictive role in the pathogenesis of glomerulosclerosis. TGF-β is a chemoattractant for fibroblasts and stimulates fibroblast proliferation and synthesis of extracellular matrix (ECM) proteins in epithelial cells. While TGF-β directly reduces the activity of metalloproteinases, which cause ECM protein degradation, it also increases the effect of metalloproteinase tissue inhibitors and indirectly inhibits ECM degradation [15]. Lim et al. [16] in their study on children with NS who received CsA treatment, as a result of the evaluation of OPN levels and renal biopsy findings before and after CsA treatment, has

shown that OPN releases increased with renal microvascular damage caused by the nephrotoxic effect of CsA. Takeda et al. [17] demonstrated that glomerular crescent, glomerulosclerosis and glomerular OPN expression were significantly decreased in rats in which they induced crescentic glomerulonephritis with anti-GBM antibodies. Yang et al. [18] found that MMF was not effective on CsA toxicity in rats that used MMF treatment in interstitial fibrosis after CsA. A significant decrease in creatinine clearance was detected after 10 weeks with CsA treatment. In the experimental models, MMF has been shown to inhibit collagen storage, proliferation in renal cells and production of TGF-β1, TNF alpha, interferon-gamma [19, 20]. In this study, similar to previous experimental studies, GSI and TDS were detected in the group with the highest CsA, and this is the detection of CsA nephrotoxicity. The IFS was found to be significantly higher in the MMF group compared to the combined therapy group. TGF-β was significantly higher in the MMF group compared to the CsA group. OPN was significantly lower in the CsA group than the other treatment groups. All these results are quite different from other studies. However, it will be possible to explain the reason for this incompatibility, based on the assumption that the death of rats in the other groups and these rats were not included in the study, the results of staining with IFS, OPN and TGF-β of the dead rats could be higher.

The most important limitation of this study is the excessive rat losses, and therefore the early termination of the study before the planned time. In addition, the presence of inconsistent data in baseline values is a possible technical error that cannot be detected.

As a result, the high loss of rats in CsA and combined therapy groups can be explained by the higher toxicity in these groups. The absence of any rat loss in the MMF-only group roughly suggests that no significant toxicity was observed with MMF. According to the results obtained, it can be said that while MMF and low dose CsA show similar successful effects in protecting serum protein and creatinine, it can be said that oxidative stress can be kept lower with low dose CsA, and hyperlipidemia can be suppressed more successfully with combined therapy. Furthermore, in this study, unlike previous studies, CsA was shown to cause glomerulosclerosis and renal damage without increasing OPN and TGF-β release. It has not been shown that MMF reduces TGF-β release and fibrosis. Moreover, low-dose CsA and combined therapy were found to be more effective in maintaining systolic and diastolic blood pressure. It is thought that by keeping the doses of cyclosporine A and MMF lower, more clear results can be obtained with a long-term study.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

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References

1. Moudgil A, Bagga A, Jordan SC. Mycophenolate mofetil therapy in frequently relapsing steroid-dependent and steroid-resistant nephrotic syndrome of childhood: current status and future directions. Pediatr Nephrol. 2005; 20(10):1376-81. DOI: 10.1007/s00467-005-1964-z.

2. Gellermann J, Querfeld U. Frequently relapsing nephrotic syndrome: treatment with mycophenolate mofetil. Pediatr Nephrol. 2004; 19(1):101-4. DOI: 10.1007/s00467-003-1300-4.

3. Huraib SO, Qureshi JI, Quadri HK, Flaiw AA, Ghamdi GA, Jumani A, et al. Mycophenolat Mofetil (MMF) efficacy in glomerunefhritis a retrospective analysis. Saudi J Kidney Dis Transpl. 2005; 16(1):23-8.

4. Mendizabal S, Zamora I, Berbel O, Sanahuja MJ, Fuentes J, Simon J. Mycophenolat mofetil in steroid/cyclosporine-dependent/ resistant nephrotic syndrome. Pediatr Nephrol 2005; 20 (16):914-9. DOI: 10.1007/s00467-005-1877-x.

5. Hagar HH, El Etter E, Arafa M. Taurine attenuates hypertension and renal dysfunction induced by cyclosporine a in rats. Clin Exp Pharmacol Physiol. 2006; 33(3):189-96. DOI: 10.111/j.1440-1681.2006.04345.x.

6. Takeda S, Takahashi M, Sado Y, Takeuchi K, Hakamata Y, Shimizu H, et al. Prevention of glomerular crescent formation in glomerulonephritis by mycophenolate mofetil in rats. Nephrol Dial Transplant. 2004; 19:2228-36. DOI: 10.1093/ndt/gfh302.

7. Medrano SA, Presas VJ, Clave PL, Masferrer MJ, Domenech JC. Efficacy and safety of combined cyclosporin A and mycophenolate mofetil therapy in patients with cyclosporin- resistant focal segmental glomerulosclerosis. Nefrologia. 2011;31(3):286-91. DOI: 10.3265/Nefrologia.pre2011.Feb.10870.

8. Gellermann J, Ehrich JHH, Qerfeld U. Sequental maintenance therapy with cyclosporin A andmycophenolate mofetil for sustained remissionof childhood steroid- resistant nephrotic syndrome. Nephrol Dial Transplant 2012; 27(5):1970-8. DOI: 10.1093/ndt/gfr572.

9. Aizawa- Yashiro T, Tsuruga K, Watanabe S, Oki E, Ito E, Tanaka H. Novel multidrug therapy for children with cyclosporine- rezistant or –intolerant nephrotic syndrome. Pediatr Nephrol. 2011; 26: 1255-61. DOI: 10.1007/s00467-011-1876-z.

10. Okuda S, Oh Y, Tsuruda H, Onoyama K, Fujimi S, Fujishima M. Adriamycin-induced nephropathy as a model of chronic progressive glomerular disease. Kidney Int. 1986; 29(2):502-10. DOI: 10.1038/ki.1986.28.

11. Akman S, Kalay S, Akkaya B, Koyun M, Akbaş H, Baysal YE, et al. Beneficial effect of triple treatment plus immunoglobulin in experimental nephrotic syndrome. Pediatr Nephrol. 2009; 24(6): 1173-80. DOI: 10.1007/s00467-009-1117-x.

12. Erisir S, Akbas H, Koyun M, Akman S. The efficiency of intraperitoneal high-dose immunoglobulin in experimental nephrotic syndrome. Pediatr Nephrol. 2006; 21(1):39-45. DOI: 10.1007/s00467-005-2046-y.

13. Zoja C, Corna D, Camozzı D, Cattaneo D, Rotalli D, Batani C, et al. How to Fully Protect the Kidney in a Severe Model of Progressive Nephropathy: A Multidrug Approach. J Am Soc Nephrol. 2002; 13:2898–908. DOI: 10.1097/01.asn.0000034912.55186.ec.

14. Blume C, Heise G, Hess A. Different effect of Cyclosporin A and mycophenolate mofetil on passive heymann nephritis in the rat. Nephron Exp Nephrol 2005; 100(2):104-12. DOI: 10.1159/000085029.

15. Border WA, Noble NA. Cytokines in kidney disease; the role of transforming growth factor-b. Am J Kidney Dis. 1993; 22(1):105-13. DOI: 10.1016/s0272-6386(12)70175-0.

16. Lim BJ, Kim PK, Hong SW, Jeong HJ. Osteopontin expression and microvascular injury in cyclosporine nephrotoxicity. Pediatr Nephrol. 2004; 19(3):288-94. DOI: 10.1007/s00467-003-1386-8.

17. Takeda S, Takahashi M, Sado Y, Takeuchi K, Hakamata Y, Shimizu H, et al. Prevention of glomerular crescent formation in glomerulonephritis by mycophenolate mofetil in rats. Nephrol Dial Transplant. 2004; 19:2228-36. DOI: 10.1093/ndt/gfh302.

18. Yang CW, Ahn HJ, Kim WY, Li C, Kim HW, Choi BS, et al. Cyclosporine withdrawal and mycophenolate mofetil treatment effects on progression of chronic cyclosporine nephrotoxicity. Kidney Int. 2002; 62(1):20-30. DOI: 10.1046/j.1523-1755.2002.00400.x.

19. Morath C, Schwenger V, Beimler J, Mehrabi A, Schmidt J, Zeier M, et al. Antifibrotic actions of mycophenolic acid. Clin Transplant 2006; 20(17):25–9. DOI: 10.1111/j1399-0012.2006.00597.x.

20. Ulinski T, Dubourg L, Saïd MH, Parchoux B, Ranchin B, Cochat P. Switch from cyclosporine A to mycophenolate mofetil in nephrotic children. Pediatr Nephrol. 2005; 20(4):482–5. DOI: 10.1007/s00467-004-1778-4.

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